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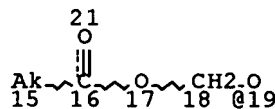
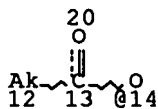
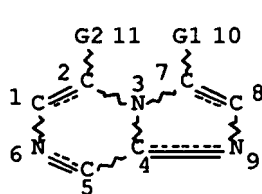
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FILE COVERS 1907 - 30 Apr 2007 VOL 146 ISS 19
 FILE LAST UPDATED: 29 Apr 2007 (20070429/ED)

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This file contains CAS Registry Numbers for easy and accurate substance identification.

=> d que 16
 L3 STR



VAR G1=14/19
 VAR G2=H/ME/I-PR
 NODE ATTRIBUTES:
 CONNECT IS E1 RC AT 12
 CONNECT IS E1 RC AT 15
 DEFAULT MLEVEL IS ATOM
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
 RING(S) ARE ISOLATED OR EMBEDDED
 NUMBER OF NODES IS 21

STEREO ATTRIBUTES: NONE
 L5 8 SEA FILE=REGISTRY SSS FUL L3
 L6 4 SEA FILE=HCAPLUS ABB=ON PLU=ON L5

=> d l6 ibib abs hitstr tot

L6 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2007 ACS on STN
 ACCESSION NUMBER: 2005:397579 HCAPLUS Full-text

DOCUMENT NUMBER: 143:419154
 TITLE: Chemical studies of fish bioluminescence
 AUTHOR(S): Kakoi, Hisae; Okada, Kunisuke
 CORPORATE SOURCE: Faculty of Pharmacy, Meijo University, Tempaku-ku,
 Nagoya, 468-8503, Japan
 SOURCE: ITE Letters on Batteries, New Technologies & Medicine
 (2005), 6(1), 38-45
 CODEN: ILBMF9; ISSN: 1531-2046
 PUBLISHER: ITE Inc.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Watasenia preluciferin (I), first isolated from the squid Watasenia scintillans, is a compound that plays a key role in the light emitting process of various bioluminescent marine organisms such as squids, shrimps, coelenterates, and fish. In the case of luminous fish, a well-known species is Myctophiformes and Stomiiformes especially the deep-sea photophores-possessing Myctophiformes fish (lantern fish), which is one of the most common and widely distributed luminous fish living in Suruga Bay and all throughout the Sea of Enshu and Kumano. Compound I was isolated either from the liver of Neoscopelus microchir (in Japanese, Sango-iwashi) or from a pair of large nasal photophores from Diaphus gigas (in Japanese, Suito-hadaka) while it was found neither in the photophores of N. microchir nor in the liver of D. gigas. On the other hand, a luciferase active toward Oplophorus luciferin (=Watasenia preluciferin) I was extracted from the flesh of D. gigas, whereas no luciferase active toward I or Cypridina luciferin was found in N. microchir. Later, a new type of bound form of I was isolated from the liver of D. gigas and the structure was established as Diaphus luciferyl β -glucopyranosiduronic acid (II) on the basis of the spectral data and chemical evidence, and by synthesis starting from I. This compound II was also detected in the liver of Diaphus watasei (in Japanese, Hadaka-iwashi) and other examined Myctophiformes fish, but not in the liver of N. microchir. It is uncertain as to which system is more favorable for the fish bioluminescence, however, as far as I is concerned, the Diaphus bioluminescent system is comparable to that of Watasenia or Oplophorus, and not to that of Cypridina as previously observed by Tsuji et al. in 1971.

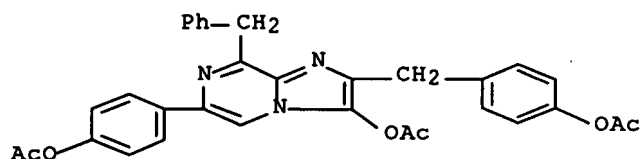
IT 65417-16-5P

RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)

(intermediate in preparation of luciferyl β -glucopyranosiduronic acid)

RN 65417-16-5 HCAPLUS

CN Imidazo[1,2-a]pyrazin-3-ol, 6-[4-(acetyloxy)phenyl]-2-[[4-(acetyloxy)phenyl]methyl]-8-(phenylmethyl)-, acetate (ester) (9CI) (CA INDEX NAME)



REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2007 ACS on STN
 ACCESSION NUMBER: 2003:376823 HCAPLUS Full-text

DOCUMENT NUMBER: 138:365147
 TITLE: Compositions, methods and kits pertaining to luminescent compounds
 INVENTOR(S): Wood, Keith; Hawkins, Erika; Scurria, Mike; Klaubert, Dieter
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 60 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003040100	A1	20030515	WO 2002-US34972	20021101
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003153090	A1	20030814	US 2001-53482	20011102
CA 2462506	A1	20030515	CA 2002-2462506	20021101
AU 2002363424	A1	20030519	AU 2002-363424	20021101
EP 1451155	A1	20040901	EP 2002-802815	20021101
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
CN 1612860	A	20050504	CN 2002-826677	20021101
JP 2005515977	T	20050602	JP 2003-542146	20021101
PRIORITY APPLN. INFO.:			US 2001-53482	A 20011102
			WO 2002-US34972	W 20021101

OTHER SOURCE(S): MARPAT 138:365147

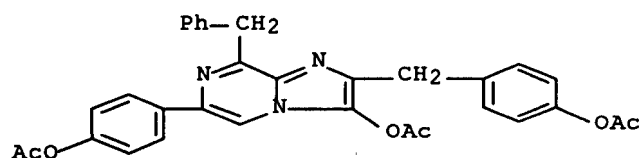
AB A method of measuring the enzymic activity of a luciferase includes contacting a luminogenic protein, such as a luciferase, with a protected luminophore to form a composition; and detecting light produced from the composition. The protected luminophore provides increased stability and improved signal-to-background ratios relative to the corresponding unmodified coelenterazine.

IT **65417-16-5P 524066-91-9P 524066-92-0P**
524066-93-1P 524066-94-2P 524066-95-3P
524066-96-4P

RL: ARU (Analytical role, unclassified); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation)
 (comps., methods and kits pertaining to luminescent compds.)

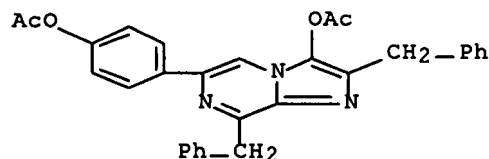
RN 65417-16-5 HCAPLUS

CN Imidazo[1,2-a]pyrazin-3-ol, 6-[4-(acetyloxy)phenyl]-2-[[4-(acetyloxy)phenyl]methyl]-8-(phenylmethyl)-, acetate (ester) (9CI) (CA INDEX NAME)



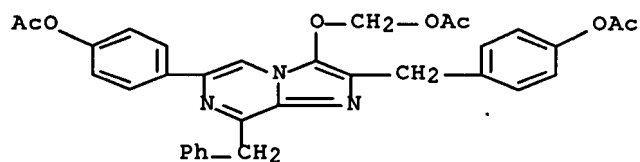
RN 524066-91-9 HCAPLUS

CN Imidazo[1,2-a]pyrazin-3-ol, 6-[4-(acetyloxy)phenyl]-2,8-bis(phenylmethyl)-, acetate (ester) (9CI) (CA INDEX NAME)



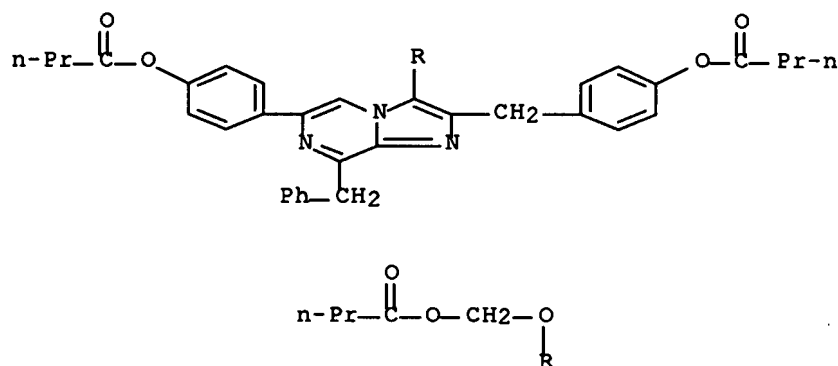
RN 524066-92-0 HCAPLUS

CN Phenol, 4-[3-[(acetyloxy)methoxy]-2-[[4-(acetyloxy)phenyl]methyl]-8-(phenylmethyl)imidazo[1,2-a]pyrazin-6-yl]-, acetate (ester) (9CI) (CA INDEX NAME)



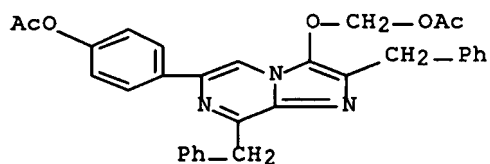
RN 524066-93-1 HCAPLUS

CN Butanoic acid, 4-[3-[(1-oxobutoxy)methoxy]-2-[[4-(1-oxobutoxy)phenyl]methyl]-8-(phenylmethyl)imidazo[1,2-a]pyrazin-6-yl]phenyl ester (9CI) (CA INDEX NAME)



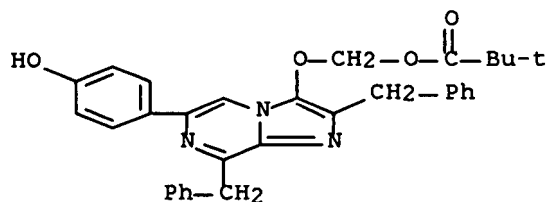
RN 524066-94-2 HCAPLUS

CN Phenol, 4-[3-[(acetyloxy)methoxy]-2,8-bis(phenylmethyl)imidazo[1,2-a]pyrazin-6-yl]-, acetate (ester) (9CI) (CA INDEX NAME)



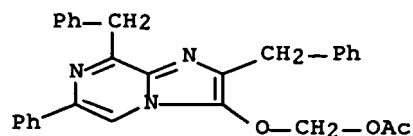
RN 524066-95-3 HCAPLUS

CN Propanoic acid, 2,2-dimethyl-, [[6-(4-hydroxyphenyl)-2,8-bis(phenylmethyl)imidazo[1,2-a]pyrazin-3-yl]oxy]methyl ester (9CI) (CA INDEX NAME)



RN 524066-96-4 HCAPLUS

CN Methanol, [[6-phenyl-2,8-bis(phenylmethyl)imidazo[1,2-a]pyrazin-3-yl]oxy]-, acetate (ester) (9CI) (CA INDEX NAME)



REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2003:108790 HCAPLUS Full-text

DOCUMENT NUMBER: 139:129758

TITLE: Coelenterazine derivatives for improved solution solubility

AUTHOR(S): Hawkins, Erika M.; O'Grady, Michael; Klaubert, Dieter; Scurria, Michael; Good, Troy; Stratford, Cathy; Flemming, Rod; Simpson, Dan; Wood, Keith V.

CORPORATE SOURCE: Promega Corporation, Madison, WI, 53715, USA

SOURCE: Bioluminescence & Chemiluminescence: Progress & Current Applications, [Proceedings of the Symposium on Bioluminescence and Chemiluminescence], 12th, Cambridge, United Kingdom, Apr. 5-9, 2002 (2002), 149-152. Editor(s): Stanley, Philip E.; Kricka, Larry J. World Scientific Publishing Co. Pte. Ltd.:

Singapore, Singapore.

CODEN: 69DPGZ; ISBN: 981-238-156-2

DOCUMENT TYPE:

Conference

LANGUAGE:

English

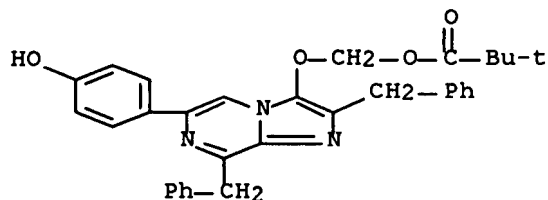
AB Intracellular luminescent techniques requiring coelenterazine, such as bioluminescence resonance energy transfer (BRET), calcium detection, and intracellular reporter measurements, must accommodate the poor stability of this substrate in physiol. buffered solns. Coelenterazine degradation leads both to loss of luminescence over time, and increased background luminescence caused by enzyme-independent oxidation (autoluminescence). Both conditions limit luminescence sensitivity by reducing the signal-to-noise ratio. Coelenterazine can be stabilized by derivatizing the enol oxygen with an acyl oxymethyl ether. This prevents spontaneous oxidation in solution while making the substrate available intracellularly upon cleavage of the blocking group by endogenous esterases. We will describe the stability of pivaloyl oxymethyl coelenterazine-h (POM coelenterazine-h), and the effect of POM coelenterazine-h on intracellular luminescence, autoluminescence, and luminescent reaction kinetics. Also, we will present the characteristics of two other coelenterazine derivs.

IT 524066-95-3D, diacetyl derivative 566945-96-8

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(coelenterazine derivs. for improved solution solubility)

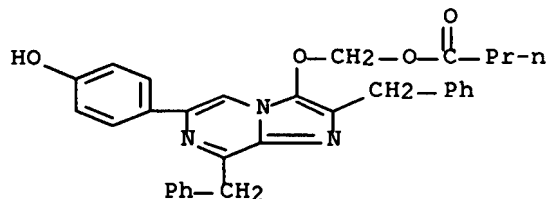
RN 524066-95-3 HCAPLUS

CN Propanoic acid, 2,2-dimethyl-, [[6-(4-hydroxyphenyl)-2,8-bis(phenylmethyl)imidazo[1,2-a]pyrazin-3-yl]oxy]methyl ester (9CI) (CA INDEX NAME)



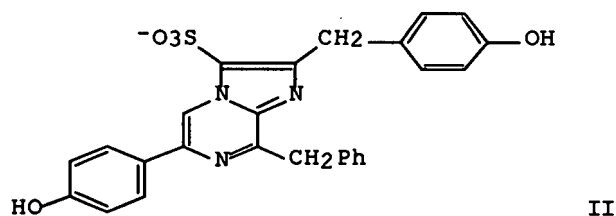
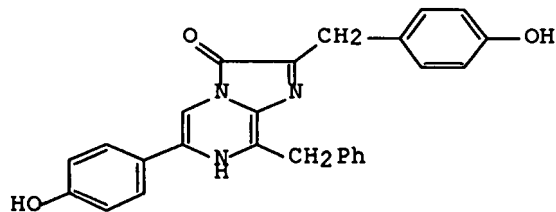
RN 566945-96-8 HCAPLUS

CN Butanoic acid, [[6-(4-hydroxyphenyl)-2,8-bis(phenylmethyl)imidazo[1,2-a]pyrazin-3-yl]oxy]methyl ester (9CI) (CA INDEX NAME)



L6 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1978:50764 HCAPLUS Full-text

DOCUMENT NUMBER: 88:50764
 TITLE: Complete structure of Renilla luciferin and luciferyl sulfate
 AUTHOR(S): Inoue, Shoji; Kakoi, Hisae; Murata, Mikiko; Goto, Toshio; Shimomura, Osamu
 CORPORATE SOURCE: Fac. Pharm., Meijo Univ., Nagoya, Japan
 SOURCE: Tetrahedron Letters (1977), (31), 2685-8
 CODEN: TELEAY; ISSN: 0040-4039
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 GI



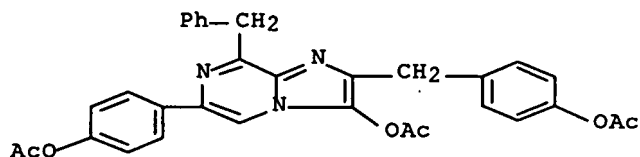
AB Examination of Renilla exts. showed that Renilla luciferin is coelenterazine (I). The structure of natural luciferyl sulfate was determined as II by comparison of natural and synthetic II. II was synthesized from I by sequential treatment with (AcO)₂O, MeOH/NH₃, and pyridine-SO₃ complex and hydrolysis with MeOH/NaOH.

IT **65417-16-5P**

RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
 (preparation and hydrolysis of)

RN 65417-16-5 HCAPLUS

CN Imidazo[1,2-a]pyrazin-3-ol, 6-[4-(acetyloxy)phenyl]-2-[[4-(acetyloxy)phenyl]methyl]-8-(phenylmethyl)-, acetate (ester) (9CI) (CA INDEX NAME)



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FILE CONTENT: 1961-PRESENT VOL 146 ISS 18 (20070427/ED)

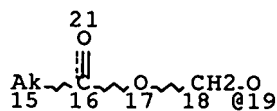
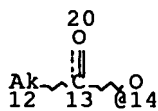
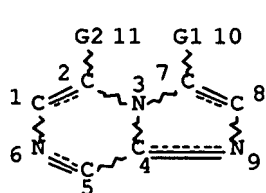
SOME MARPAT RECORDS ARE DERIVED FROM INPI DATA FOR 1961-1987

MOST RECENT CITATIONS FOR PATENTS FROM MAJOR ISSUING AGENCIES
 (COVERAGE TO THESE DATES IS NOT COMPLETE):

US 2007060644 15 MAR 2007
 DE 102006023116 15 MAR 2007
 EP 1762248 14 MAR 2007
 JP 2007059877 08 MAR 2007
 WO 2007030662 15 MAR 2007
 GB 2429975 14 MAR 2007
 FR 2890657 16 MAR 2007
 RU 2295953 27 MAR 2007
 CA 2556850 24 FEB 2007

Expanded G-group definition display now available.

=> d que l16
 L3 STR



VAR G1=14/19
 VAR G2=H/ME/I-PR
 NODE ATTRIBUTES:
 CONNECT IS E1 RC AT 12
 CONNECT IS E1 RC AT 15
 DEFAULT MLEVEL IS ATOM
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
 RING(S) ARE ISOLATED OR EMBEDDED
 NUMBER OF NODES IS 21

STEREO ATTRIBUTES: NONE
 L5 8 SEA FILE=REGISTRY SSS FUL L3
 L6 4 SEA FILE=HCAPLUS ABB=ON PLU=ON L5
 L15 2 SEA FILE=MARPAT SSS FUL L3
 L16 1 SEA FILE=MARPAT ABB=ON PLU=ON L15 NOT L6

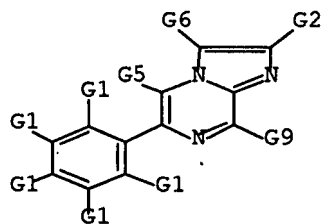
=> d l16 ibib abs qhit tot

L16 ANSWER 1 OF 1 MARPAT COPYRIGHT 2007 ACS on STN
 ACCESSION NUMBER: 135:371764 MARPAT Full-text
 TITLE: Preparation of aminopyrazines and imidazolopyrazinones as
 antioxidants
 INVENTOR(S): Marchand-Brynaert, Jacqueline; Cavalier,
 Jean-Francois; Rees, Jean-Francois; De Tollenaere,
 Catherine; Burton, Maggi
 PATENT ASSIGNEE(S): Universite Catholique de Louvain, Belg.
 SOURCE: PCT Int. Appl., 57 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2001087853	A1	20011122	WO 2001-EP5588	20010516
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1292580	A1	20030319	EP 2001-943383	20010516
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2004034225	A1	20040219	US 2003-276398	20030728
PRIORITY APPLN. INFO.:				
			EP 2000-870107	20000517
			EP 2000-870293	20001212
			WO 2001-EP5588	20010516

OTHER SOURCE(S): CASREACT 135:371764

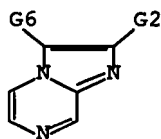
AB Antioxidants, 5 2-amino-(p-hydroxyphenyl)pyrazines and 3 (p-hydroxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-ones were prepared and claimed useful in diagnostic procedures, as food additives, polymer additives and as UV screens in cosmetics. E.g., 2-amino-3,5- dibromopyrazine was treated with p-methoxyphenylboronic acid in the presence of bis(benzonitrile)palladium dichloride and 1,4- bis(diphenylphosphino)butane in a solvent mix of EtOH, aqueous sodium carbonate and toluene to give 66% 2-amino-3,5-bis(p-methoxyphenyl)pyrazine, which was demethylated with EtSNa in DMF to give 88% 2-amino-3,5-bis(p-hydroxyphenyl)pyrazine (I). In tests on inhibition of lipid peroxidn. 2-aminopyrazines possessing 2 aryl substituents, one of them being a p-hydroxyphenyl in o- or p- position with respect to the amino group, are endowed with antioxidative properties. However, the p-hydroxyphenyl conferred more activity when located at position 5 than at position 3. The presence of p-hydroxyphenyl groups at both positions 3 and 5 as in I produced a very active compound. Analogs lacking the free phenol groups showed reduced activities. Corresponding imidazolopyrazinones combined the properties of both the imidazolopyrazinones (delay of the onset of peroxidn.) and the aminopyrazines (lower rate of oxidation after onset).



G6 = OCOMe
 Patent location:
 Note:
 Stereochemistry:

claim 1
 or prodrugs, or pharmaceutically acceptable
 addition salts, or tautomerically isomeric forms
 or stereochemically isomeric forms

MSTR 3



G6 = OCOMe
 Patent location:
 Note:
 Stereochemistry:

claim 27
 or prodrugs, or pharmaceutically acceptable
 addition salts, or tautomerically isomeric forms
 or stereochemically isomeric forms

REFERENCE COUNT:

10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

INVENTOR NAME SEARCH

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L17 2610 SEA WOOD K/AU OR WOOD K ?/AU OR WOOD KEITH?/AU
 L18 948 SEA HAWKINS E/AU OR HAWKINS E ?/AU OR HAWKINS ERI!A?/AU
 L19 34 SEA ("SCURRIA M"/AU OR "SCURRIA M A"/AU OR "SCURRIA M S"/AU OR
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 L20 198 SEA ("KLAUBERT D"/AU OR "KLAUBERT D H"/AU OR "KLAUBERT D K"/AU
 OR "KLAUBERT DIETER"/AU OR "KLAUBERT DIETER H"/AU OR "KLAUBERT
 DIETER HEINZ"/AU)
 L21 44 SEA (L17 AND (L18 OR L19 OR L20)) OR (L18 AND (L19 OR L20)) OR
 (L19 AND L20)

=> dup rem l21

PROCESSING COMPLETED FOR L21

L22 24 DUP REM L21 (20 DUPLICATES REMOVED)
 ANSWERS '1-15' FROM FILE HCAPLUS
 ANSWERS '16-23' FROM FILE BIOSIS
 ANSWER '24' FROM FILE WPIX

=> d l22 ibib ab tot

L22 ANSWER 1 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1
 ACCESSION NUMBER: 2006:1279865 HCAPLUS Full-text
 DOCUMENT NUMBER: 146:57589
 TITLE: Luminogenic and fluorogenic compounds and methods to
 detect molecules or conditions
 INVENTOR(S): Daily, William; **Hawkins, Erika;**
Klaubert, Dieter; Liu, Jianquan; Meisenheimer,
 Poncho; **Scurria, Michael;** Shultz, John W.;
 Unch, James; **Wood, Keith V.;** Zhou, Wenhui;
 Valley, Michael P.; Cali, James J.
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 328pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2006130551	A2	20061207	WO 2006-US20731	20060530
WO 2006130551	A8	20070201		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
US 2007015790	A1	20070118	US 2006-444145	20060531
PRIORITY APPLN. INFO.:			US 2005-685957P	P 20050531
			US 2005-693034P	P 20050621
			US 2005-692925P	P 20050622
			US 2006-790455P	P 20060407

OTHER SOURCE(S): MARPAT 146:57589

AB A method to detect the presence or amount of at least one mol. in a sample which employs a derivative of luciferin or a derivative of a fluorophore is provided.

L22 ANSWER 2 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2006:1161664 HCAPLUS Full-text

DOCUMENT NUMBER: 146:116710

TITLE: Electrophilic aromatic substituted luciferins as bioluminescent probes for glutathione S-transferase assays

AUTHOR(S): Zhou, Wenhui; Shultz, John W.; Murphy, Nancy; **Hawkins, Erika M.**; Bernad, Laurent; Good, Troy; Moothart, Leonard; Frackman, Susan; **Klaubert, Dieter H.**; Bulleit, Robert F.; **Wood, Keith V.**

CORPORATE SOURCE: Promega Biosciences Inc., San Luis Obispo, CA, 93401, USA

SOURCE: Chemical Communications (Cambridge, United Kingdom) (2006), (44), 4620-4622

CODEN: CHCOFS; ISSN: 1359-7345

PUBLISHER: Royal Society of Chemistry

DOCUMENT TYPE: Journal

LANGUAGE: English

AB New highly sensitive latent bioluminescent luciferin substrates were designed and synthesized for monitoring mammalian glutathione S-transferase (GST) and Schistosoma japonicum enzyme activities.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 3 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2006:137803 HCAPLUS Full-text

DOCUMENT NUMBER: 144:384691

TITLE: New Bioluminogenic Substrates for Monoamine Oxidase Assays

AUTHOR(S): Zhou, Wenhui; Valley, Michael P.; Shultz, John;
Hawkins, Erika M.; Bernad, Laurent; Good,
Troy; Good, Dave; Riss, Terry L.; **Klaubert,**
Dieter H.; **Wood, Keith V.**

CORPORATE SOURCE: Promega Biosciences Inc., San Luis Obispo, CA, 93401,
USA

SOURCE: Journal of the American Chemical Society (2006),
128(10), 3122-3123
CODEN: JACSAT; ISSN: 0002-7863

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Novel bioluminogenic substrates were designed for probing monoamine oxidase (MAO) activity based on a simple and effective β -elimination strategy. By modifying the amino group and the central core of luciferin derivs., we have developed a series of substrates useful for assays of MAO A or B, or both. One of these substrates, exhibiting low Km values and high signal-to-background ratios with both isoenzymes, was shown to accurately measure the Ki values of known MAO inhibitors. This substrate is a key component in the development of a highly sensitive homogeneous MAO assay for high-throughput screening (HTS) of compds. in drug discovery and for monitoring MAO activity in complex biol. systems. This design strategy should be applicable to fluorogenic MAO substrates and could broaden the structural requirements of substrates for other enzyme assays.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 4 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 2006:1268397 HCAPLUS Full-text

DOCUMENT NUMBER: 146:200635

TITLE: A bioluminescent assay for monoamine oxidase activity

AUTHOR(S): Valley, Michael P.; Zhou, Wenhui; **Hawkins, Erika M.**; Shultz, John; Cali, James J.; Worzella, Tracy; Bernad, Laurent; Good, Troy; Good, Dave; Riss, Terry L.; **Klaubert, Dieter H.**; **Wood, Keith V.**

CORPORATE SOURCE: Promega Corporation, Madison, WI, 53711, USA

SOURCE: Analytical Biochemistry (2006), 359(2), 238-246
CODEN: ANBCA2; ISSN: 0003-2697

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal

LANGUAGE: English

AB This article describes a novel two-step homogeneous bioluminescent assay for monoamine oxidase (MAO) that is simple, sensitive, and amenable to high-throughput screening. In the first step, MAO reacts with an aminopropylether analog of Me ester luciferin. In the second step, a luciferin detection reagent inactivates MAO and converts the product of the first step into a luminescent signal. The amount of light produced is proportional to the amount of MAO and the time of incubation in the first step, but the luminescent signal is stable in the second step with a half-life greater than 5 h. The assay has high precision, is more sensitive than current fluorescent methods, and can accurately measure the binding consts. of known substrates and inhibitors. An automated screen of the Sigma-RBI Library of Pharmacol. Active Compds. (LOPAC1280) revealed a surprisingly high percentage of MAO inhibitors (16%) with a low false hit rate (0.9%). This implies that a significant number of compds. interact with the MAO enzymes and suggests that it is important to include MAO assays in drug metabolism studies. Other advantages of this bioluminescent assay over comparable fluorescent assays are discussed.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 5 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5
 ACCESSION NUMBER: 2005:1292638 HCAPLUS Full-text
 DOCUMENT NUMBER: 144:33522
 TITLE: Substrate-binding, catalytically inactive hydrolases
 as carriers for the immobilization of fusion proteins
 INVENTOR(S): Darzins, Aldis; Encell, Lance; Johnson, Tonny;
Klaubert, Dieter; Los, Georgyi V.; Mcdougall,
 Mark; **Wood, Keith V.**; Wood, Monika G.;
 Zimprich, Chad
 PATENT ASSIGNEE(S): USA
 SOURCE: U.S. Pat. Appl. Publ., 121 pp., Cont.-in-part of U.S.
 Ser. No. 768,976.
 CODEN: USXXCO
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005272114	A1	20051208	US 2004-6031	20041206
US 2004214258	A1	20041028	US 2004-768976	20040130
US 2006024808	A1	20060202	US 2005-194110	20050729
CA 2575611	A1	20060908	CA 2005-2575611	20050729
WO 2006093529	A2	20060908	WO 2005-US27307	20050729
WO 2006093529	A3	20070322		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
US 2007087400	A1	20070419	US 2006-509796	20060824
PRIORITY APPLN. INFO.:			US 2003-444094P	P 20030131
			US 2003-474659P	P 20030530
			US 2004-768976	A2 20040130
			US 2004-592499P	P 20040730
			US 2004-6031	A 20041206
			WO 2005-US27307	W 20050729

OTHER SOURCE(S): MARPAT 144:33522

AB Hydrolase variants that retain substrate binding, and capable of forming a covalent bond with a substrate, but lacking the catalytic activity to release the hydrolysis products are described for use in the immobilization of proteins onto surfaces carrying a substrate for the hydrolase are described. The binding of the hydrolase to substrate is more stable than that of the wild type enzyme. The catalytically inactive variant has at least two amino acid substitutions. Substrates for hydrolases comprising one or more functional groups are also provided, as well as methods of using the mutant hydrolase and the substrates of the invention. Also provided is a fusion protein capable of forming a stable bond with a substrate and cells which express the fusion protein. Development of a catalytically inactive variant of the haloalkane dehalogenase of *Rhodococcus rhodochrous* is demonstrated. Use of fusion

products with fluorescent proteins and enzymes in imaging in vivo are demonstrated.

L22 ANSWER 6 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6

ACCESSION NUMBER: 2005:291435 HCAPLUS Full-text

DOCUMENT NUMBER: 143:341532

TITLE: Homogeneous, bioluminescent protease assays: Caspase-3 as a model

AUTHOR(S): O'Brien, Martha A.; Daily, William J.; Hesselberth, P. Eric; Moravec, Richard A.; **Scurria, Michael A.**; **Klaubert, Dieter H.**; Bulleit, Robert F.; **Wood, Keith V.**

CORPORATE SOURCE: Promega Corporation, Madison, WI, USA

SOURCE: Journal of Biomolecular Screening (2005), 10(2), 137-148

CODEN: JBISF3; ISSN: 1087-0571

PUBLISHER: Sage Publications

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Using caspase-3 as a model, the authors have developed a strategy for highly sensitive, homogeneous protease assays suitable for high-throughput, automated applications. The assay uses peptide-conjugated aminoluciferin as the protease substrate and a firefly luciferase that has been molecularly evolved for increased stability. By combining the proluminescent caspase-3 substrate, Z-DEVD-aminoluciferin, with a stabilized luciferase in a homogeneous format, the authors developed an assay that is significantly faster and more sensitive than fluorescent caspase-3 assays. The assay has a single-step format, in which protease cleavage of the substrate and luciferase oxidation of the aminoluciferin occurs simultaneously. Because these processes are coupled, they rapidly achieve steady state to maintain stable luminescence for several hours. Maximum sensitivity is attained when this steady state occurs; consequently, this coupled-enzyme system results in a very rapid assay. The homogeneous format inherently removes trace contamination by free aminoluciferin, resulting in extremely low background and yielding exceptionally high signal-to-noise ratios and excellent Z' factors. Another advantage of a luminescent format is that it avoids problems of cell autofluorescence or fluorescence interference that can be associated with synthetic chemical and natural product libraries. This bioluminescent, homogeneous format should be widely applicable to other protease assays.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 7 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 7

ACCESSION NUMBER: 2005:150211 HCAPLUS Full-text

TITLE: Analytical biotechnology

AUTHOR(S): **Wood, Keith V.**; **Klaubert, Dieter H.**

CORPORATE SOURCE: Promega Corporation, Madison, WI, 53711, USA

SOURCE: Current Opinion in Biotechnology (2005), 16(1), 1-2

CODEN: CUOBE3; ISSN: 0958-1669

PUBLISHER: Elsevier Ltd.

DOCUMENT TYPE: Journal; Editorial

LANGUAGE: English

AB Unavailable

L22 ANSWER 8 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 2004:698252 HCAPLUS Full-text

DOCUMENT NUMBER: 141:187324

TITLE: Methods and kits for dual enzymatic assays whereby

light is quenched from luminescent reactions
 INVENTOR(S): **Hawkins, Erika**; Butler, Braeden; **Wood, Keith V.**
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 77 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004072299	A1	20040826	WO 2004-US4075	20040212
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2004210982	A1	20040826	AU 2004-210982	20040212
CA 2515217	A1	20040826	CA 2004-2515217	20040212
US 2004224377	A1	20041111	US 2004-777461	20040212
EP 1592805	A1	20051109	EP 2004-710594	20040212
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2006517413	T	20060727	JP 2006-503513	20040212
PRIORITY APPLN. INFO.:				
			US 2003-447065P	P 20030212
			WO 2004-US4075	W 20040212

AB The present invention relates to single and dual reporter luminescence assays utilizing reagents to quench an optical, e.g., an enzyme-mediated luminescence, reaction. In one embodiment of the invention, a reagent is added to an assay which selectively quenches a first enzyme-mediated luminescence reaction without affecting a subsequent distinct enzyme-mediated luminescent reaction(s). An assay kit containing one or more selective quench reagents, and compns. comprising the quench reagent(s), are also provided.

L22 ANSWER 9 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9

ACCESSION NUMBER: 2004:698213 HCAPLUS Full-text

DOCUMENT NUMBER: 141:221282

TITLE: Mutant Rhodococcus dehalogenase and functionalized chloroalkane substrates useful for covalent tethering of functional groups to proteins

INVENTOR(S): **Wood, Keith V.**; Los, Georgyi V.; Bulleit, Robert F.; **Klaubert, Dieter**; Mcdougall, Mark; Zimprich, Chad

PATENT ASSIGNEE(S): Promega Corporation, USA

SOURCE: PCT Int. Appl., 185 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004072232	A2	20040826	WO 2004-US2607	20040130

WO 2004072232 A9 20041014
 WO 2004072232 A3 20050127
 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
 CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
 GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
 LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI
 RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE,
 BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU,
 MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN,
 GQ, GW, ML, MR, NE, SN, TD, TG
 AU 2004211584 A1 20040826 AU 2004-211584 20040130
 CA 2514564 A1 20050726 CA 2004-2514564 20040130
 EP 1594962 A2 20051116 EP 2004-707032 20040130
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
 CN 1764721 A 20060426 CN 2004-80008194 20040130
 PRIORITY APPLN. INFO.: US 2003-444094P P 20030131
 US 2003-474659P P 20030530
 WO 2004-US2607 W 20040130

OTHER SOURCE(S): MARPAT 141:221282

AB A mutant hydrolase optionally fused to a protein of interest is provided. Thus, Rhodococcus haloalkane dehalogenase DhaA with His-272 substituted with Phe is capable of forming a bond with a chloroalkane substrate for the corresponding nonmutant (wild-type) hydrolase which is more stable than the bond formed between the wild-type hydrolase and the substrate. The chloroalkane substrate contains a functional group which binds Ca²⁺ or K⁺, or Na⁺, is pH sensitive, is a radionuclide, is electron opaque, is a chromophore or fluorophore, is a MRI contrast agent, is a substance that fluoresces in the presence of NO, or is sensitive to reactive oxygen. Substrates for hydrolases comprising one or more functional groups are synthesized comprising TAMRA-, FAM-, and ROX.5-Cl₄H₂4O₄-Cl or biotin-Cl₈H₃2O₄-Cl, as methods of using the mutant DhaA and the substrates of the invention for cell imaging in vivo are provided. Mutant Staphylococcus aureus β -lactamase (blaZ)-based tethering of functional groups is also demonstrated. Also provided is a fusion protein capable of forming a stable bond with a substrate and cells which express the fusion protein.

L22 ANSWER 10 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 10

ACCESSION NUMBER: 2004:570131 HCAPLUS Full-text

DOCUMENT NUMBER: 141:119301

TITLE: Improving the accuracy of luciferase-based assays for high throughput screening by using tolerance enhancement agents

INVENTOR(S): **Hawkins, Erika**; Cali, James J.; Ho, Samuel
 Kin Sang; O'Brien, Martha; Somberg, Richard; Bulleit,
 Robert F.; **Wood, Keith V.**

PATENT ASSIGNEE(S): Promega Corporation, USA

SOURCE: PCT Int. Appl., 68 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004059294	A2	20040715	WO 2003-US41454	20031223
WO 2004059294	A3	20060629		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,			

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
 PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN,
 TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
 BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE,
 ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK,
 TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
 CA 2508072 A1 20040715 CA 2003-2508072 20031223
 AU 2003300008 A1 20040722 AU 2003-300008 20031223
 US 2005026171 A1 20050203 US 2003-746995 20031223
 EP 1588143 A2 20051026 EP 2003-800272 20031223
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
 JP 2006517401 T 20060727 JP 2005-510073 20031223
 PRIORITY APPLN. INFO.: US 2002-436173P P 20021223
 US 2003-444264P P 20030131
 US 2003-447334P P 20030213
 WO 2003-US41454 W 20031223

AB The invention concerns methods and kits for improving the accuracy of luciferase-based assays for high throughput screening of compound libraries by reducing the number of false hits. A method and kit is provided for enhancing the tolerance of an assay reagent to compds. in an assay sample, the assay reagent including a luciferase enzyme. The method includes contacting the luciferase with a tolerance enhancement agent in an amount sufficient to substantially protect luciferase enzyme activity from interference of the compound and minimize interference by at least about 10% relative to an assay not having tolerance enhancement agent. Tolerance-enhancing effect of detergents on the inhibition of luciferase was studied. Minimization of false hit occurrence using tolerance enhancement agents such as detergents was demonstrated.

L22 ANSWER 11 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 11
 ACCESSION NUMBER: 2004:270174 HCAPLUS Full-text
 DOCUMENT NUMBER: 140:299425
 TITLE: Luminescent cytochrome P 450 assay using luciferase, luciferin derivatives and pyrophosphatase, and drug screening applications
 INVENTOR(S): Cali, James J.; **Klaubert, Dieter**; Daily, William; Ho, Samuel Kin Sang; Frackman, Susan; **Hawkins, Erika; Wood, Keith V.**
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 130 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004027378	A2	20040401	WO 2003-US29078	20030916
WO 2004027378	A3	20041125		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
 PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN,

TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
 KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
 FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
 BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
 CA 2497560 A1 20040401 CA 2003-2497560 20030916
 AU 2003267245 A1 20040408 AU 2003-267245 20030916
 EP 1546162 A2 20050629 EP 2003-749715 20030916
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
 JP 2006508339 T 20060309 JP 2004-537859 20030916
 US 2004171099 A1 20040902 US 2003-665314 20030919
 PRIORITY APPLN. INFO.: US 2002-412254P P 20020920
 US 2003-483309P P 20030627
 WO 2003-US29078 W 20030916

OTHER SOURCE(S): MARPAT 140:299425

AB The present invention provides methods, compns., substrates, and kits useful for analyzing the metabolic activity in cells, tissue, and animals and for screening test compds. for their effect on cytochrome P 450 activity. In particular, a one-step and two-step methods using luminogenic mols., e.g. luciferin or coelenterazines, that are cytochrome P 450 substrates and that are also bioluminescent enzyme, e.g., luciferase, pro-substrates are provided. Upon addition of the luciferin derivative or other luminogenic mol. into a P 450 reaction, the P 450 enzyme metabolizes the mol. into a bioluminescent enzyme substrate, e.g., luciferin and/or luciferin derivative metabolite, in a P 450 reaction. The resulting metabolite(s) serves as a substrate of the bioluminescent enzyme, e.g., luciferase, in a second light-generating reaction. Luminescent cytochrome P 450 assays with low background signals and high sensitivity are disclosed and isoform selectivity is demonstrated. The present invention also provides an improved method for performing luciferase reactions which employs added pyrophosphatase to remove inorg. pyrophosphate, a luciferase inhibitor which may be present in the reaction mixture as a contaminant or may be generated during the reaction. The present method further provides a method for stabilizing and prolonging the luminescent signal in a luciferase-based assay using luciferase stabilizing agents such as reversible luciferase inhibitors.

L22 ANSWER 12 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 12
 ACCESSION NUMBER: 2003:633682 HCAPLUS Full-text
 DOCUMENT NUMBER: 139:193612
 TITLE: Bioluminescent protease assay using aminoluciferin
 linked to peptide substrate and luciferase
 INVENTOR(S): O'Brian, Martha; **Wood, Keith; Klaubert,**
Dieter; Daily, Bill
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 55 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003066611	A1	20030814	WO 2003-US2936	20030131
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,			

PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
 UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
 KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
 FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF,
 BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA 2474695	A1	20030814	CA 2003-2474695	20030131
AU 2003216139	A1	20030902	AU 2003-216139	20030131
US 2003211560	A1	20031113	US 2003-356665	20030131
US 7148030	B2	20061212		
EP 1472238	A1	20041103	EP 2003-737580	20030131

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK

JP 2005530485	T	20051013	JP 2003-565985	20030131
US 2006183177	A1	20060817	US 2006-346043	20060202
US 2006121546	A1	20060608	US 2006-347054	20060203

PRIORITY APPLN. INFO.:

US 2002-353158P	P	20020201
US 2003-356665	A1	20030131
WO 2003-US2936	W	20030131

AB A sensitive bioluminescent assay to detect proteases including caspases, trypsin and tryptase is provided. The method comprises contacting a sample suspected of having one or more caspases with a mixture comprising beetle luciferase and an aminommodified beetle aminoluciferin or a carboxyterminal protected derivative thereof, wherein the amino group of aminoluciferin or the derivative thereof is modified so as to covalently link a substrate for the caspase via a peptide bond to aminoluciferin or the carboxyterminal protected derivative thereof. If the sample comprises a caspase having a recognition site in the substrate, the substrate is cleaved at the peptide bond that links the substrate to aminoluciferin, yielding aminoluciferin, a substrate for the luciferase, in the mixture Luminescence is then detected. The method further comprises correlating luminescence with protease concentration or activity, i.e., increased luminescence correlates with increased protease concentration or activity. Also provided is a compound comprising aminoluciferin or a carboxyterminal protected derivative thereof covalently linked via a peptide bond to a protease recognition site such as a caspase recognition site, a trypsin recognition site, or a tryptase recognition site. A specific compound of the invention is a compound of formula I (R = peptide with an aspartic acid, lysine, or arginine C-terminus; R' = H, carboxy protecting group, e.g., Cl-6-alkyl, Ph, benzyl ester, counterion). The invention also provides synthetic processes and intermediates disclosed herein, which are useful for preparing compds. of the invention. As described herein below, using a substrate for caspase 3 and 7 that was linked to either aminoluciferin or rhodamine-110, it was found that the limit of detection for the aminoluciferin-based substrate was 0.2 to 0.5 μ U of purified caspase while that for the rhodamine-110-based substrate was 10 μ U. As also described herein, it was found that the limit of detection of caspase expressing cells with the aminoluciferin-based substrate was 15 cells at 1 h while the limit of detection for the rhodamine-110-based substrate was 150 cells at 1 h.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 13 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 13

ACCESSION NUMBER: 2003:376823 HCAPLUS Full-text

DOCUMENT NUMBER: 138:365147

TITLE: Compositions, methods and kits pertaining to luminescent compounds

INVENTOR(S): **Wood, Keith; Hawkins, Erika; Scurria, Mike; Klaubert, Dieter**

PATENT ASSIGNEE(S): Promega Corporation, USA

SOURCE: PCT Int. Appl., 60 pp.

CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003040100	A1	20030515	WO 2002-US34972	20021101
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003153090	A1	20030814	US 2001-53482	20011102
CA 2462506	A1	20030515	CA 2002-2462506	20021101
AU 2002363424	A1	20030519	AU 2002-363424	20021101
EP 1451155	A1	20040901	EP 2002-802815	20021101
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
CN 1612860	A	20050504	CN 2002-826677	20021101
JP 2005515977	T	20050602	JP 2003-542146	20021101
PRIORITY APPLN. INFO.:			US 2001-53482	A 20011102
			WO 2002-US34972	W 20021101

OTHER SOURCE(S): MARPAT 138:365147

AB A method of measuring the enzymic activity of a luciferase includes contacting a luminogenic protein, such as a luciferase, with a protected luminophore to form a composition; and detecting light produced from the composition. The protected luminophore provides increased stability and improved signal-to-background ratios relative to the corresponding unmodified coelenterazine.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 14 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 14
 ACCESSION NUMBER: 2001:924094 HCAPLUS Full-text
 DOCUMENT NUMBER: 136:50649
 TITLE: Method for increasing luminescence assay sensitivity
 INVENTOR(S): **Hawkins, Erika**; Centanni, John M.; Sankbeil, Jacqueline; **Wood, Keith V.**
 PATENT ASSIGNEE(S): Promega Corporation, USA
 SOURCE: PCT Int. Appl., 45 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096862	A2	20011220	WO 2001-US18363	20010607
WO 2001096862	A3	20020718		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,				

RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,
 VN, YU, ZA, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
 DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
 BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
 US 7118878 B1 20061010 US 2000-590884 20000609
 CA 2411179 A1 20011220 CA 2001-2411179 20010607
 EP 1297337 A2 20030402 EP 2001-942027 20010607
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
 JP 2004503777 T 20040205 JP 2002-510941 20010607
 US 2004096924 A1 20040520 US 2003-692587 20031024
 US 7078181 B2 20060718
 US 2006051827 A1 20060309 US 2004-991759 20041118
 US 7108996 B2 20060919
 PRIORITY APPLN. INFO.: US 2000-590884 A 20000609
 WO 2001-US18363 W 20010607
 US 2003-692587 A3 20031024

AB A method for increasing the sensitivity of a luminescent assay comprising carrying out the assay in the presence of an organic compound that reduces luminescence that is not dependent on the presence of an analyte by at least about 10 fold, and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold.

L22 ANSWER 15 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2003:108790 HCAPLUS Full-text

DOCUMENT NUMBER: 139:129758

TITLE: Coelenterazine derivatives for improved solution solubility

AUTHOR(S): **Hawkins, Erika M.**; O'Grady, Michael;
Klaubert, Dieter; **Scurria, Michael**;
 Good, Troy; Stratford, Cathy; Flemming, Rod; Simpson, Dan; **Wood, Keith V.**

CORPORATE SOURCE: Promega Corporation, Madison, WI, 53715, USA

SOURCE: Bioluminescence & Chemiluminescence: Progress & Current Applications, [Proceedings of the Symposium on Bioluminescence and Chemiluminescence], 12th, Cambridge, United Kingdom, Apr. 5-9, 2002 (2002), 149-152. Editor(s): Stanley, Philip E.; Kricka, Larry J. World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore.
 CODEN: 69DPGZ; ISBN: 981-238-156-2

DOCUMENT TYPE: Conference

LANGUAGE: English

AB Intracellular luminescent techniques requiring coelenterazine, such as bioluminescence resonance energy transfer (BRET), calcium detection, and intracellular reporter measurements, must accommodate the poor stability of this substrate in physiol. buffered solns. Coelenterazine degradation leads both to loss of luminescence over time, and increased background luminescence caused by enzyme-independent oxidation (autoluminescence). Both conditions limit luminescence sensitivity by reducing the signal-to-noise ratio. Coelenterazine can be stabilized by derivatizing the enol oxygen with an acyl oxymethyl ether. This prevents spontaneous oxidation in solution while making the substrate available intracellularly upon cleavage of the blocking group by endogenous esterases. We will describe the stability of pivaloyl oxymethyl coelenterazine-h (POM coelenterazine-h), and the effect of POM coelenterazine-h on intracellular luminescence, autoluminescence, and luminescent reaction kinetics. Also, we will present the characteristics of two other coelenterazine derivs.

L22 ANSWER 16 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2007:78327 BIOSIS Full-text
DOCUMENT NUMBER: PREV200700078755
TITLE: Bioluminescent protease assay.
AUTHOR(S): Anonymous; O'Brien, Martha [Inventor]; **Wood, Keith V.** [Inventor]; **Klaubert, Dieter** [Inventor];
Daily, William [Inventor]
CORPORATE SOURCE: Madison, WI USA
ASSIGNEE: Promega Corporation
PATENT INFORMATION: US 07148030 20061212
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (DEC 12 2006)
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jan 2007
Last Updated on STN: 24 Jan 2007
AB A sensitive bioluminescent assay to detect proteases including caspases,
trypsin and tryptase is provided.

L22 ANSWER 17 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2007:39141 BIOSIS Full-text
DOCUMENT NUMBER: PREV200700045138
TITLE: Method for increasing luminescence assay sensitivity.
AUTHOR(S): Anonymous; **Hawkins, Erika** [Inventor]; Centanni,
John M. [Inventor]; Sankbeil, Jacqueline [Inventor];
Wood, Keith V. [Inventor]
CORPORATE SOURCE: Madison, WI USA
ASSIGNEE: Promega Corporation
PATENT INFORMATION: US 07118878 20061010
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (OCT 10 2006)
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Jan 2007
Last Updated on STN: 3 Jan 2007
AB The invention provides kits and methods for increasing the sensitivity of a
bio-luminescent assay, which employ an organic compound that, for instance,
reduces luminescence that is not dependent on the presence of an analyte by at
least about 10 fold and reduces luminescence that is dependent on the presence
of an analyte by less than about 7 fold, reduces luminescence generated by
luminogenic molecules not bound to an enzyme by at least about 10 fold and
reduces the luminescence generated by luminogenic molecules bound to an enzyme
by less than about 7 fold, or reduces autoluminescence by at least about 10
fold and reduces luminescence that is dependent on the presence of an analyte
by less than about 7 fold.

L22 ANSWER 18 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2007:23679 BIOSIS Full-text
DOCUMENT NUMBER: PREV200700032812
TITLE: Method for increasing luminescence assay sensitivity.
AUTHOR(S): Anonymous; **Hawkins, Erika** [Inventor]; Centanni,

John M. [Inventor]; Sankbeil, Jacqueline [Inventor];
Wood, Keith V. [Inventor]
CORPORATE SOURCE: Madison, WI USA
ASSIGNEE: Promega Corporation
PATENT INFORMATION: US 07108996 20060919
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (SEP 19 2006)
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006
AB A method for increasing the sensitivity of a bio-luminescent assay comprising
carrying out the assay in the presence of an organic compound that reduces
luminescence that is not dependent on the presence of an analyte by at least
about 10 fold, and that reduces, maintains, or increases the luminescence that
is dependent on the presence of an analyte.

L22 ANSWER 19 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2006:669792 BIOSIS Full-text
DOCUMENT NUMBER: PREV200600682021
TITLE: Kits for increasing luminescence assay sensitivity.
AUTHOR(S): Anonymous; **Hawkins, Erika** [Inventor]; Centanni,
John M. [Inventor]; Sankbeil, Jacqueline [Inventor];
Wood, Keith V. [Inventor]
CORPORATE SOURCE: Madison, WI USA
ASSIGNEE: Promega Corporation
PATENT INFORMATION: US 07078181 20060718
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (JUL 18 2006)
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Dec 2006
Last Updated on STN: 6 Dec 2006

AB Assay kits for increasing the sensitivity of luminescent assays are provided
that include an organic compound that reduces luminescence that is not
dependent on the presence of an analyte by at least about 10 fold, and that
reduces, maintains, or increases the luminescence that is dependent on the
presence of an analyte.

L22 ANSWER 20 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2006:584734 BIOSIS Full-text
DOCUMENT NUMBER: PREV200600595360
TITLE: Homogeneous, bioluminescent assays for proteasome activity.
AUTHOR(S): O'Brien, Martha A. [Reprint Author]; Moravec, Richard A.;
Riss, Terry; **Scurria, Michael A.**; Daily, William
J.; **Klaubert, Dieter H.**; **Wood, Keith V.**
; Bulleit, Robert F.
CORPORATE SOURCE: Promega Corp, Madison, WI USA
SOURCE: Proceedings of the American Association for Cancer Research
Annual Meeting, (APR 2006) Vol. 47, pp. 321.
Meeting Info.: 97th Annual Meeting of the
American-Association-for-Cancer-Research (AACR).
Washington, DC, USA. April 01 -05, 2006. Amer Assoc Canc
Res.

ISSN: 0197-016X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Nov 2006
Last Updated on STN: 8 Nov 2006

L22 ANSWER 21 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2007:261852 BIOSIS Full-text
DOCUMENT NUMBER: PREV200700271919
TITLE: A multipurpose fusion protein tag for analysis of dynamic
cellular events.
AUTHOR(S): Learish, Randall D. [Reprint Author]; Los, Georgyi V.;
Zimprich, Chad; McDougall, Mark; Karassina, Natasha; Nath,
Nidhi; Darzins, Al; **Klaubert, Dieter**; Bulleit,
Robert F.; **Wood, Keith**
CORPORATE SOURCE: Promega Corp, Madison, WI USA
SOURCE: Proceedings of the American Association for Cancer Research
Annual Meeting, (APR 2005) Vol. 46, No. Suppl. S, pp. 902.
Meeting Info.: 96th Annual Meeting of the
American-Association-for-Cancer-Research. Anaheim, CA, USA.
April 16 -20, 2005. Amer Assoc Canc Res.
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Apr 2007
Last Updated on STN: 25 Apr 2007

L22 ANSWER 22 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2006:219506 BIOSIS Full-text
DOCUMENT NUMBER: PREV200600222633
TITLE: The HaloTag (TM): a novel technology for cellular analysis.
AUTHOR(S): Los, G. V. [Reprint Author]; Zimprich, C.; McDougall, M.
G.; Karassina, N.; Learish, R.; **Klaubert, D. H.**;
Darzins, A.; Bulleit, R. F.; **Wood, K.**
CORPORATE SOURCE: Promega Corp, Madison, WI USA
SOURCE: Journal of Neurochemistry, (JUN 2005) Vol. 94, No. Suppl.
1, pp. 15.
Meeting Info.: 36th Annual Meeting of the
American-Society-for-Neurochemistry. Madison, WI, USA. June
25 -29, 2005. Amer Soc Neurochem.
CODEN: JONRA9. ISSN: 0022-3042.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Apr 2006
Last Updated on STN: 5 Apr 2006

L22 ANSWER 23 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on
STN

ACCESSION NUMBER: 2004:195934 BIOSIS Full-text
DOCUMENT NUMBER: PREV200400196493
TITLE: Site - specific localization of small molecule reporters
within living cells.
AUTHOR(S): Los, G. V. [Reprint Author]; Zimprich, C. [Reprint Author];
McDougall, M. G.; Karassina, N. [Reprint Author]; Learish,
R. [Reprint Author]; **Klaubert, D.**; **Wood,**

CORPORATE SOURCE: **K.** [Reprint Author]; Bulleit, B. [Reprint Author]
 R&D, Promega Corp., Madison, WI, USA
 SOURCE: Society for Neuroscience Abstract Viewer and Itinerary
 Planner, (2003) Vol. 2003, pp. Abstract No. 232.5.
<http://sfn.scholarone.com>. e-file.
 Meeting Info.: 33rd Annual Meeting of the Society of
 Neuroscience. New Orleans, LA, USA. November 08-12, 2003.
 Society of Neuroscience.
 DOCUMENT TYPE: Conference; (Meeting)
 Conference; Abstract; (Meeting Abstract)
 LANGUAGE: English
 ENTRY DATE: Entered STN: 14 Apr 2004
 Last Updated on STN: 14 Apr 2004
 AB The ability to specifically label proteins can help reveal information about
 protein functions and dynamics within the complex biochemical environment of
 the living cells. Here we describe a novel technology for covalently
 tethering functional groups ((FG), e.g. fluorescent dye) to a universal
 reporting protein (URP) within cells. The URP is a mutant enzyme capable of
 forming a stable covalent bond to a modified substrate coupled to the
 functional group. In our initial approach the URP is a halo-alkane
 dehalogenase from *Rhodococcus rhodochrous* (DhaA) with a mutation in a critical
 active site residue. The activity of DhaA cleaves carbon-halogen bonds in
 aliphatic and aromatic halogenated compounds involving a typical hydrolytic
 triad. In this reaction an enzyme-substrate complex is formed by a
 nucleophilic attack involving Asp106 and the formation of an ester
 intermediate; His272 activates H₂O that hydrolyzes this intermediate releasing
 product from the catalytic center. A point mutation in DhaA involving a
 substitution of phenylalanine for His272 impairs the hydrolysis step leading
 to a stable covalent intermediate with substrate and any conjugated FG. This
 technology allows the labeling of mutant DhaA or mutant DhaA fusion proteins
 expressed in cells. A significant advantage of this approach is the
 flexibility to create labeled proteins with a potentially wide range of
 optical properties or other functionalities. The technology can be applied in
 different cells and organisms allowing a variety of experimental approaches to
 study protein function in living cells.

L22 ANSWER 24 OF 24 WPIX COPYRIGHT 2007 THE THOMSON CORP on STN
 ACCESSION NUMBER: 2006-135434 [14] WPIX
 CROSS REFERENCE: 2004-635192; 2006-044483
 DOC. NO. CPI: C2006-046443 [14]
 TITLE: New mutant dehalogenase comprising at least two amino
 acid substitutions relative to a corresponding wild-type
 dehalogenase, useful for isolating, detecting,
 identifying, imaging, displaying, or localizing molecules
 of interest
 DERWENT CLASS: B04; D16
 INVENTOR: DARZINS A; ENCELL L; **KLAUBERT D**; LOS GEORGYI V;
 MCDOUGALL M; **WOOD K V**; WOOD M G; ZIMPRICH C
 PATENT ASSIGNEE: (DARZ-I) DARZINS A; (ENCE-I) ENCELL L; (KLAU-I) KLAUBERT
 D; (LGEO-I) LOS GEORGYI V; (MCDO-I) MCDOUGALL M; (WOOD-I)
 WOOD K V; (WOOD-I) WOOD M G; (ZIMP-I) ZIMPRICH C
 COUNTRY COUNT: 1
 PATENT INFO ABBR.:

PATENT NO	KIND DATE	WEEK	LA	PG	MAIN IPC
US 20060024808	A1	20060202	(200614)*	EN	170[60]

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 20060024808	A1 Provisional	US 2004-592499P	20040730
US 20060024808	A1	US 2005-194110	20050729

PRIORITY APPLN. INFO: US 2005-194110 20050729
 US 2004-592499P 20040730

AB US 20060024808 A1 UPAB: 20060227

NOVELTY - A mutant dehalogenase comprising at least two amino acid substitutions relative to a corresponding wild-type dehalogenase, where the mutant dehalogenase forms a bond with a dehalogenase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type dehalogenase and the substrate, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a method for detecting or determining the presence or amount of a mutant hydrolase;
- (2) a method for isolating a molecule, cell or sub-cellular organelle of interest in a sample;
- (3) a method for labeling a cell;
- (4) a method for labeling a cell;
- (5) a polynucleotide encoding the mutant hydrolase;
- (6) a mutant hydrolase comprising at least two amino acid substitutions relative to a corresponding wild-type hydrolase, where one substitution is at a position corresponding to amino acid residue 272 of a Rhodococcus rhodochrous dehalogenase or at a position corresponding to amino acid residue 106 of a Rhodococcus rhodochrous dehalogenase, and a second substitution is at an amino acid residue corresponding to position 175, 176 or 273 of a Rhodococcus rhodochrous dehalogenase; and
- (7) a thermostable mutant dehalogenase comprising at least one substitution at a position corresponding to amino acid residue 175 of a Rhodococcus rhodochrous dehalogenase, which substitution is correlated with enhanced thermostability relative to a corresponding mutant dehalogenase without the substitution at the position corresponding to amino acid residue 175; and a compound of formula XXIX-XXXIV.

USE - The mutant dehalogenase is useful for isolating, detecting, identifying, imaging, displaying, or localizing molecules of interest; labeling cells, including live cell imaging; or labeling proteins in vitro and/or in vivo.

SEARCH HISTORY

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(FILE 'HOME' ENTERED AT 10:29:26 ON 30 APR 2007)

FILE 'HCAPLUS' ENTERED AT 10:29:29 ON 30 APR 2007

E US2001-053482/APPS

L1 1 SEA ABB=ON PLU=ON (US2001-53482/AP OR US2001-53482/PRN)
SEL RN

FILE 'REGISTRY' ENTERED AT 10:30:06 ON 30 APR 2007

L2 11 SEA ABB=ON PLU=ON (50909-86-9/BI OR 524066-91-9/BI OR
524066-92-0/BI OR 524066-93-1/BI OR 524066-94-2/BI OR 524066-95
-3/BI OR 524066-96-4/BI OR 55779-48-1/BI OR 61869-41-8/BI OR
65417-16-5/BI OR 70217-82-2/BI)

FILE 'REGISTRY' ENTERED AT 10:30:41 ON 30 APR 2007

L3 STR
L4 0 SEA SSS SAM L3
L5 8 SEA SSS FUL L3

FILE 'HCAPLUS' ENTERED AT 10:33:01 ON 30 APR 2007

L6 4 SEA ABB=ON PLU=ON L5

FILE 'BEILSTEIN' ENTERED AT 10:33:14 ON 30 APR 2007

L7 0 SEA SSS SAM L3
L8 1 SEA SSS FUL L3
L9 1 SEA ABB=ON PLU=ON L8 AND RN/FA
L10 0 SEA ABB=ON PLU=ON L8 AND BABSAN/FA
SEL RN L8
D COST

FILE 'REGISTRY' ENTERED AT 10:34:25 ON 30 APR 2007

L11 1 SEA ABB=ON PLU=ON 65417-16-5/RNX
L12 1 SEA ABB=ON PLU=ON L11 AND L2
L13 1 SEA ABB=ON PLU=ON L11 AND L5

FILE 'MARPAT' ENTERED AT 10:35:26 ON 30 APR 2007

L14 0 SEA SSS SAM L3
L15 2 SEA SSS FUL L3
L16 1 SEA ABB=ON PLU=ON L15 NOT L6

FILE 'HCAPLUS, MEDLINE, EMBASE, BIOSIS, DISSABS, WPIX' ENTERED AT 10:36:23 ON 30 APR 2007

L17 2610 SEA ABB=ON PLU=ON WOOD K/AU OR WOOD K ?/AU OR WOOD KEITH?/AU

L*** DEL 938 S HAWKINS E/AU OR HAWKINS E ?/AU OR HAWKINS ERI!A/AU
L18 948 SEA ABB=ON PLU=ON HAWKINS E/AU OR HAWKINS E ?/AU OR HAWKINS
ERI!A?/AU
E SCURRIA M/AU

L19 34 SEA ABB=ON PLU=ON ("SCURRIA M"/AU OR "SCURRIA M A"/AU OR
"SCURRIA M S"/AU OR "SCURRIA MICHAEL"/AU OR "SCURRIA MICHAEL
A"/AU OR "SCURRIA MIKE"/AU)
E KLAUBERT D/AU

L20 198 SEA ABB=ON PLU=ON ("KLAUBERT D"/AU OR "KLAUBERT D H"/AU OR
"KLAUBERT D K"/AU OR "KLAUBERT DIETER"/AU OR "KLAUBERT DIETER
H"/AU OR "KLAUBERT DIETER HEINZ"/AU)

L21 44 SEA ABB=ON PLU=ON (L17 AND (L18 OR L19 OR L20)) OR (L18 AND

(L19 OR L20)) OR (L19 AND L20)

FILE 'HCAPLUS' ENTERED AT 10:45:16 ON 30 APR 2007

D QUE L6

D L6 IBIB ABS HITSTR TOT

FILE 'MARPAT' ENTERED AT 10:45:32 ON 30 APR 2007

D QUE L16

D L16 IBIB ABS QHIT TOT

FILE 'HCAPLUS, MEDLINE, EMBASE, BIOSIS, DISSABS, WPIX' ENTERED AT
10:46:23 ON 30 APR 2007

D QUE

L22

24 DUP REM L21 (20 DUPLICATES REMOVED)

ANSWERS '1-15' FROM FILE HCAPLUS

ANSWERS '16-23' FROM FILE BIOSIS

ANSWER '24' FROM FILE WPIX

D L22 IBIB AB TOT